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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :		(11) International Publication Number:
C12P 21/06, C12Q 1/68 C07K 3/00, 13/00, 15/00 C07K 17/00, G01N 33/566	A1	WO 93/06229
		(43) International Publication Date: 1 April 1993 (01.04.93)

(21) International Application Number: PCT/US92/07641

(22) International Filing Date: 14 September 1992 (14.09.92)

(30) Priority data:
759,568 13 September 1991 (13.09.91) US

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(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, SE).

Published
With international search report.

(54) Title: CLONING OF cDNA ENCODING A FUNCTIONAL HUMAN INTERLEUKIN-8 RECEPTOR

(57) Abstract

A cDNA clone from HL60 neutrophils, designated p2, whichencodes a human interleukin-8 receptor. This IL-8 receptor can be expressed in oocytes or transfected host cells. This receptor has 77 % amino acid identity with a second human neutrophil receptor isotype that also binds IL-8. It also exhibits 69 % amino acid identity with a protein reported to be an N-formyl peptide receptor from rabbit neutrophils.

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CLONING OF cDNA ENCODING A FUNCTIONAL HUMAN INTERLEUKIN-8 RECEPTOR

BACKGROUND OF THE INVENTION

Field of the Invention

The invention relates to identification and characterization of a human interleukin-8 receptor which also binds gro. In another aspect, it relates to stable expression of functionally active IL-8 receptor in host cells.

Background Information

Stimulation of neutrophils with IL-8, NAP-2 or gro causes mobilization of intracellular calcium stores and elicits motile, secretory, and metabolic responses that are critical to the role of the neutrophil in host defenses. See B. Moser, I. Clark-Lewis, R. Zwahlen, M. Baggiolini, J. Exp. Med. 171, 1797 (1990); A. Walz, B. Dewald, V. von Tscharner, M. Baggiolini, ibid. 170, 1745 (1989); and M. Thelen et al., FASEB J. 2, 2702 (1988). IL-8 is an inflammatory cytokine that activates neutrophil chemotaxis, degranulation and the respiratory burst, the means by which neutrophils attack pathogens in the body. Neutrophils express receptors for IL-8 that are coupled to guanine nucleotide binding proteins (G-prot ins); binding of IL-8 t its receptor induc s th mobilization of intracellular calcium st res. IL-8, also kn wn as

neutrophil activating protein-1 or NAP-1, is a potent chemoattractant for neutrophils that is produced by many cell types in response to inflammatory stimuli. See J. J. Oppenheim, Prog. Clin. Biol. Res. 349, 405 (1990). This IL-8 receptor has 77% amino acid identity with a second human neutrophil receptor isotype that also binds IL-8 (Genentech, FASEB, April 1991).

IL-8 is structurally and functionally related to several members of the macrophage inflammatory protein-2 (or MIP-2) family of cytokines. These include MIP-2, gro (or melanoma growth-stimulatory activity), and NAP-2. See S. D. Wolpe and A. Cerami, FASEB J. 3, 2565 (1989); B. Moser, I. Clark-Lewis, R. Zwahlen, M. Baggiolini, J. Exp. Med. 171, 1797 (1990); and A. Walz, B. Dewald, V. von Tscharner, M. Baggiolini, ibid. 170, 1745 (1989). High affinity binding sites for IL-8 have been found on transformed myeloid precursor cells such as HL60 and THP-1 as well as on neutrophils. See B. Moser, C. Schumacher, V. von Tscharner, I. Clark-Lewis, M. Baggiolini, J. Biol. Chem. 266, 10666 (1991); J. Besemer, A. Hujber, B. Kuhn, J. Biol. Chem. 264, 17409 (1989); P. M. Grob et al., ibid. 265, 8311 (1990); A. K. Samanta, J. J. Oppenheim, K. Matsushima, J. Exp. Med. 169, 1185 (1989); E. J. Leonard et al., J. Immunol. 144, 1323 (1989). NAP-2 and gro c mpete with IL-8 for binding t human

neutrophils suggesting that th y interact with the sam rec ptors. S e B. Moser, C. Schumach r, V. von Tscharner, I. Clark-Lewis, M. Baggiolini, J. Biol. Chem. 266, 10666 (1991).

Functional expression in the Xenopus oocyte has established the identity of cDNA clones encoding rabbit and human forms of another peptide chemoattractant receptor on neutrophils, the N-formyl peptide receptor. See K. M. Thomas, H. Y. Pyun, J. Navarro, J. Biol. Chem. 265, 20061 (1990); and F. Boulay, M. Tardif, L. Brouchon, P. Vignais, Biochem. Biophys. Res. Commun. 168, 1103 (1990). Yet the amino acid sequence of the rabbit form of the receptor (originally designated F3R) is only 28% identical with that of the human form (designated in this paper as FPR); this is far greater than the differences between species reported for all other G protein-coupled receptors. See T. I. Bonner, A. C. Young, M. R. Brann, N. J. Buckley, Neuron 1, 403 (1988); and S. Yokoyama, K. E. Eisenberg, A. F. Wright, Mol. Biol. Evol. 6, 342 (1989).

By cloning the complementary DNA sequence encoding the human interleukin 8 receptor (IL8R), the primary structure of this receptor can be established and its role in the inflammatory response can be further investigated. Such studies c uld potentially lead t the design of new anti-inflammat ry agents.

SUMMARY OF THE INVENTION

It is an object of th present invention to isolate and characterize human interleukin-8 receptors.

The present invention relates to a cDNA clone from HL60 neutrophils, designated p2, which encodes an IL-8 receptor having an amino acid sequence as shown in Figure 3.

The present invention further relates to the IL-8 receptor itself which has intracellular calcium store mobilizing properties and ligand binding properties.

Furthermore, the invention relates to an oocyte expressing IL-8 receptors produced by injecting a cRNA molecule transcribed from the cDNA clone, p2. Additionally, the present invention relates to a method of producing the IL-8 receptor in an oocyte.

Furthermore, the present invention relates to a host cell stably transfected with the cDNA clone, p2. In addition, the present invention relates to a method of producing the IL-8 receptor in a host cell.

In addition, the present invention relates to a method of detecting the presence or absence of a DNA segment encoding the IL-8 receptor in a sample by contacting the sample with a DNA probe having at least a p rtion f the sequ nce of the cDNA clone, p2.

Various other objects and advantages of the present invention will become apparent from the following figures and description of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the results of in vitro translation of p2 and distribution of p2 mRNA in various cell types.

Fig. 1 (A) shows translation of p2 cRNA by rabbit reticulocyte lysate (lane 2) compared with control lysate to which no RNA was added (lane 1). Red blood cell membrane proteins were used as molecular mass standards indicated in kilodaltons (kD) at the left. The gel was exposed to XAR-2 film for 12 hours.

Fig. 1 (B) shows p2 mRNA distribution. The blot of RNA from neutrophils was prepared separately from 10 μg of total cellular RNA. The other lanes derive from a single blot containing total cellular RNA from peripheral blood T lymphocytes activated with phytohemagglutinin (PHA-T, 5 μg), THP-1 cells (5 μg) and Jurkat cells (3 μg). The lane marked HL60 contains 10 μg of polyadenylated [poly (A)$^+$] RNA from undifferentiated HL60 cells. The arrow indicates the location of a faint band of RNA from THP-1 cells. Both blots were hybridized under identical conditions with the same p2 probe and were

washed at 68°C in 0.1 X·SSPE f r 1 h ur. Bl ts were exposed to XAR-2 film in a Quanta III cassette at -80°C for 5 days. Results with 3 ind pendent HL60 cell preps and 2 separate THP-1 and neutrophil blots were identical.

Fig. 2 shows the expression of a human IL-8 receptor in Xenopus oocytes.

Fig. 2 (A) shows signal transduction by the IL-8 receptor. Four days after injection with 5 ng of p2 cRNA, oocytes were stimulated with the indicated concentration of IL-8 and calcium efflux was measured. Data derive from five replicate determinations per point and are representative of three separate experiments.

Fig. 2 (B) shows binding of [^{125}I]-IL-8 to oocytes expressing a functional IL-8 receptor. Total (O) and non-specific binding (O) was determined by incubating oocytes injected with p2 cRNA with the indicated concentration of radioligand in the absence or presence of unlabeled IL-8 (1 μM), respectively. The data shown are the mean ± SEM of triplicate determinations per point and are representative of two separate experiments. Non-specific binding was subtracted from total binding to determine specific binding (□). C5a (1 μM) did n t displace [^{125}I]-IL-8 from oocytes injected with p2 cRNA. Specific binding of [^{125}I]-IL-8 by ocyt s injected with water was undetectable.

Fig. 2 (C) shows ligand s l ctivity of the IL-8 rec ptor. Thr e days after injection with 5 ng of p2 cRNA, oocyt s were stimulated with the indicated concentration of IL-8 (○), gro (□), NAP-2 (○), FMLP (♦) or C5a (Δ), and calcium efflux activity was measured. Data derive from eight replicate determinations per point. The response of oocytes injected with 50 ng of HL60 neutrophil RNA to FMLP (1 μM) or C5a (500 nM) was 51 ± 3 and 16 ± 5%, respectively. The response of oocytes injected with 5 ng of an irrelevant cRNA encoding the rat serotonin 1c receptor was negligible for each of the five ligands; the response to the relevant ligand, serotonin (1 μM), was 34 ± 3% (n = 6). See D. Julius, A. B. McDermott, R. Axel, T. M. Jessell, Science 241, 558 (1988). In (A) and (C) basal amounts of calcium efflux and calcium uptake were similar among all experimental conditions.

Fig. 3 shows the primary structure of a human IL-8 receptor (IL-8R) and its alignment with that of the reported rabbit (F3R) and human (FPR) N-formyl peptide receptors. Vertical bars indicate identical residues for each adjacent sequence position. Shaded boxes indicate the location of predicted membrane spanning segments I through VII as determined by the Kyte-Doolittle algorithm. See J. Kyte and R. F. D littl , J. Molec. Biol. 157, 105 (1982). Op n b x s designate pr dicted sites f r N-

linked glycosylation. Arabic numbers above the sequence blocks enumerate the IL-8 receptor sequence and are left justified. Dashes indicate gaps that were inserted to optimize the alignment. Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gly; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

Fig. 4 relates to a genomic analysis of a human IL-8 receptor. A Nytran blot of human genomic DNA digested with the indicated restriction endonucleases was hybridized with full-length cDNA of the IL-8 receptor at high stringency (final wash at 68°C in 0.1 X SSPE for 1 hour). The blot was exposed to Kodak XAR-2 film in a Quanta III cassette at -80°C for 5 days. The position of chain length standards is indicated in kilobases at the left. The autoradiogram shown is representative of two independent experiments.

Fig. 5 shows the nucleotide sequence of p2, having a length of 1510 nucleotides.

Fig. 6 shows the oligonucleotide probe corresponding to nucleotides 238 to 276 of the cDNA sequence of F3R.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention relates to a human IL-8 receptor and its encoding cDNA clone, designated p2. This receptor is a newly identified human homologue of F3R.

The present invention further relates to a cRNA molecule transcribed from the cDNA clone, p2. The present invention also relates to an interleukin-8 receptor which is also a gro receptor and has greater than 77% homology with the amino acid sequence shown in Figure 3.

Furthermore, the present invention relates to an oocyte, such as a Xenopus laevis oocyte, which expresses a functionally active form of the IL-8 receptor, when it is injected with a cRNA molecule transcribed from the cDNA clone, p2. The invention also relates to the method of injecting p2 into individual oocytes using standard techniques and experimental conditions which would be understood by one skilled in the art.

Additionally, the present invention relates to a recombinant DNA molecule and to a host cell transfected therewith which expresses a functionally active form of the IL-8 receptor. Using standard methodology well known in the art, a recombinant DNA molecule comprising a vector and a cDNA segment, p2, can be constructed using methods known in the art without undue experimentation. The transfected host

cell can be cultured, and the expressed protein can be isolated and obtained in a substantially pure form using methods known in the art. COS cells (monkey kidney cells) or NIH 3T3 or other eukaryotic host cells conventionally used in the art to express inserted cDNA may also be used. As a vector, pcDNAI or pCLNXneo can be used as well as other vectors conventionally used in the art.

The invention further relates to the IL-8 receptor's intracellular calcium store mobilizing properties and ligand binding properties. Specifically, the IL-8 receptor encoded by p2 has been shown to bind both IL-8 and gro and to exhibit calcium flux as a result of such binding.

The invention also relates to a method of detecting the presence or absence of a DNA segment encoding the IL-8 receptor or a related receptor from the MIP-2 family in a sample by contacting the sample with a p2 probe having at least a portion of the cDNA clone. The method is performed under conditions such that hybridization between the probe and the DNA segment from the sample occurs. This hybridization can be detected by assaying for the presence or absence of a complex formed between the probe and the DNA segment. The techniques and experimental conditions used would be understood by one skilled in the art.

The invention also relates to a method for screening ligands for the IL-8 receptor by measuring binding affinity and calcium flux resulting from the binding of the ligand to the receptor expressed in the oocyte or the host cell. The techniques and experimental conditions used would understood by one skilled in the art.

The invention also relates to a gene therapy treatment by which an individual with a condition relating to a deficiency of IL-8 receptor might be treated by administering to the individual DNA encoding the IL-8 receptor in a form such that the DNA would alleviate the deficiency of IL-8 receptor. Conventional gene therapy techniques understood by one skilled in the art could be used. However, there is so far no evidence which points to the existence of such a condition of IL-8 receptor deficiency.

The following non-limiting examples are provided to further describe the present invention.

EXAMPLES

Example 1

The DNA segment which encodes the IL-8 receptor was obtained in the form of the cDNA clone, p2, using the following techniques and conditions.

cDNA libraries were constructed in the vector UniZAP (Stratagene, La Jolla, CA) from 2-kb and 3.5-kb fractions of poly(A)+ RNA from HL60 neutrophils that had been separated on a sucrose gradient as described in P. M. Murphy, E. K. Gallin, H. L. Tiffany, J. Immunol. 145, 2227 (1990).

Approximately 3 X 10^5 plaque-forming units (pfu) from the 2-kb library were screened with the ^{32}P-labeled F3R oligonucleotide probe.

Both the 2-kb (3 X 10^5 pfu) and the 3.5-kb (10^6 pfu) libraries were rescreened under conditions of low stringency with a ^{32}P-labeled probe of p2 cDNA synthesized from random primers. The final wash was for one hour at 55°C in 5 X SSPE (1 X SSPE contains 150 mM NaCl, 10 mM NaH_2PO_4, and 1 mM Na_2EDTA, pH 7.4).

The DNA sequence was determined with sequence-based oligonucleotides (17 bases) by the dideoxynucleotide chain termination method. See F. Sanger, S. Nicklen, A. R. Coulson, Proc. Natl. Acad. Sci. U.S.A. 74, 5463 (1977).

DNA sequences were analyzed using software from the University of Wisconsin Genetics Computer Group on a Cray supercomputer maintained by the National Cancer Institute Advanced Scientific Computing Laboratory, Frederick Cancer Research Facility, Frederick, MD. See J. Devereux, P. Haeberli, O. Smithies, Nucleic Acids Res. 12, 389 (1984).

An oligonucleotide probe corresponding to nucleotides 238 to 276 of the cDNA sequence of F3R (Fig. 6) was hybridized to cDNA libraries made from RNA from the promyelocytic leukemia cell line HL60 grown for two days in the presence of dibutyryl cyclic adenosine monophosphate (750 μM), a treatment that induces a neutrophil-like phenotype. See K. M. Thomas, H. Y. Pyun, J. Navarro, J. Biol. Chem. 265, 20061 (1990). Seven clones that encoded an identical gene product were isolated. The longest of these, designated p2, was sequenced on both strands (Fig. 5). Confirmatory sequences were obtained from the other clones. A 1065-bp (base pair) open reading frame begins with the sequence AACATGG which conforms to the Kozak consensus criteria for translation initiation sites. See M. Kozak, Nucleic Acids Res. 15, 8125 (1987). A 24 bp poly(A) tail is found at the end of a 405 bp 3'-untranslated region.

The cDNA clone, p2, which was obtained was characterized and found to encode the IL-8 receptor.

Example 2

p2 cRNA was synthesized by in vitro transcription with T3 RNA polymerase of a pBluescript construct that had been cleaved with Xho I. p2 cRNA (500 ng) was incubated for 30 min at 30°C with rabbit reticulocyte lysate and [^{35}S]-methionin

in a 25 µl reacti n volume (Prom ga, Madison, WI). Labeled proteins (40% of the yield) were then separat d by SDS-polyacrylamide gel electrophoresis [10% gels (Novex, Encinitas, CA)]. The gel was stained with Coomassie blue, fixed, impregnated with Fluoro-Hance (Research Products International, Mount Prospect, ILL), and dried before autoradiography.

It has been found that RNA synthesized in vitro from p2 cDNA (p2 cRNA) directed the synthesis of a polypeptide of 32 kilodaltons in vitro (Fig. 1A). This is the size of the deglycosylated native N-formyl peptide receptor as well as the size found for FPR protein synthesized in vitro. See H. L. Malech, J. P. Gardner, D. F. Heiman, S. A. Rosenzweig, J. Biol. Chem. 260, 2509 (1985). It is known that binding sites for N-formyl peptides are expressed in mature but not in immature myeloid cells. See R. Sullivan, J. D. Griffin, H. L. Malech, Blood 70, 1222 (1987). It is also known that expression of RNA for FPR is restricted to mature myeloid cells as well.

In contrast, it has been found that when p2 was used as a probe, it hybridized with a single 3-kb band on blots of RNA from the myeloid precursor cell lines HL60 and THP-1, and from normal blood-derived human neutrophils, but not from peripheral blood T lymphocytes or Jurkat cells (Fig. 1B). (For a descripti n of RNA pr paration and bl t

hybridizati n, see P. M. Murphy and H. L. Tiffany, J. Biol. Chem. 265, 11615 (1990).) This pattern of expressi n f p2 RNA is m re like th distributi n of IL-8 binding sites than N-formyl peptide binding sites. See J. Besemer, A. Hujber, B. Kuhn, J. Biol. Chem. 264, 17409 (1989); P. M. Grob et al., ibid. 265, 8311 (1990); A. K. Samanta, J. J. Oppenheim, K. Matsushima, J. Exp. Med. 169, 1185 (1989); and E. J. Leonard et al., J. Immunol. 144, 1323 (1989).

Example 3

The materials and methods used for the calcium efflux assay were as described in P. M. Murphy, E. K. Gallin, H. L. Tiffany, J. Immunol. 145, 2227 (1990). Oocytes were microinjected with RNA samples in a total volume of 50 nl per oocyte 3 days after harvesting and were then incubated at 20 to 23°C for 2 to 4 days. Oocytes were then incubated with $^{111}Ca^{2+}$ [50 μCi/ml (ICN Biomedicals, Costa Mesa, CA)] for 3 hours. After ten washes with medium, individual oocytes were stimulated with ligand in wells of a 96-well tissue culture plate containing 100 μl of medium. Three 100 μl samples of the incubation medium were collected and analyzed by liquid scintillation counting: a) the final 100 μl wash (20 min) before application of ligand; b) fluid containing the stimulus, removed after a 20 min incubati n with th oocyte; and c) the cyte

s lubilized in SDS (1%) in medium 20 min after stimulati n. Data are presented as the mean ± standard error of the mean (SEM) of the percent of loaded "Ca" that was released by individual oocytes in response to the stimulus, or [(b-a) + (b+c)] X 100. FMLP and recombinant human C5a was from Sigma, St. Louis, MO. Recombinant human IL-8 was from Genzyme, Boston, MA. Recombinant human NAP-2 was from Bachem, Philadelphia, PA.

IL-8 was iodinated to a specific activity of 260 Ci/mmole as described in H. L. Malech, J. P. Gardner, D. F. Heiman, S. A. Rosenzweig, J. Biol. Chem. 260, 2509 (1985). The radioligand was qualified by binding to human neutrophils. Single oocytes were incubated with [^{125}I]-IL-8 for 30 min on ice in 10 µl binding buffer (Hanks balanced salt solution with 25 mM HEPES, 1% bovine serum albumin, pH 7.4). Unbound ligand was removed by pelleting the oocyte through 300 µl F50 silicone fluid (General Electric, Waterford, NY). The tube was quick-frozen and gamma emissions from the amputated tips were counted.

When Xenopus oocytes were injected with p2 cRNA, they mobilized intracellular calcium in response to IL-8 with an EC_{50} of 20 nM (Fig. 2A), but did not respond to N-formyl methionyl-leucyl-phenylalanine (FMLP). This valu is appr ximately 20-f ld higher than that report d for human

n utr phils with r c mbinant human IL-8. See B. Moser, I. Clark-Lewis, R. Zwahlen, M. Baggi lini, J. Exp. Med. 171, 1797 (1990). The r cept r specifically bound IL-8 over the same concentration range as for stimulation of calcium flux (Fig. 2B). Since specific binding did not saturate at the highest concentration of radioligand that could be meaningfully tested, a dissociation constant could not be determined. Thus the receptor encoded by p2, when expressed in the oocyte, appears to bind IL-8 with a lower affinity than do neutrophil binding sites for IL-8. See B. Moser, C. Schumacher, V. von Tscharner, I. Clark-Lewis, M. Baggiolini, J. Biol. Chem. 266, 10666 (1991); J. Besemer, A. Hujber, B. Kuhn, J. Biol. Chem. 264, 17409 (1989); P. M. Grob et al., ibid. 265, 8311 (1990); A. K. Samanta, J. J. Oppenheim, K. Matsushima, J. Exp. Med. 169, 1185 (1989); and E. J. Leonard et al., J. Immunol. 144, 1323 (1989).

The IL-8 receptor expressed by Xenopus oocytes also activated a calcium flux in response to structurally related ligands with a rank order of potency of IL-8 > gro > NAP-2. This is identical to the rank order of competition with [^{125}I]-IL-8 for binding to neutrophils. See B. Moser, C. Schumacher, V. von Tscharner, I. Clark-Lewis, M. Baggiolini, J. Biol. Chem. 266, 10666 (1991). C5a, a structurally unrelated chem attractant that is

similar in size (74 amino acids) and charge (pI 8.6) to IL-8, did not activate the IL-8 receptor (Fig. 2C).

To further support the data obtained from the oocyte studies, the p2 cDNA was also cloned into the vector pCDNAI. When COS cells are transiently transfected with the pCDNAI-p2 construct, specific binding sites were detected with ^{125}I-labeled IL-8 and gro protein. In contrast to the ligand binding affinity in the oocyte environment, the affinity for the ligand when the receptor encoded by p2 was expressed in the COS cell was higher. Specifically, the k_d for IL-8 was 2 nM and the k_d for gro was 1.3 nM. These results corroborate the data obtained from the oocyte studies, indicating that p2 encodes a receptor for both IL-8 and gro.

Example 4

A p2 probe was hybridized under conditions of high stringency to blots of human genomic DNA. Specifically, human genomic DNA (3 µg per lane) was digested with 6 units of Eco RI, Eco RV, Hind III, Pst I, or Xba I restriction endonucleases (Boehringer-Mannheim, Indianapolis, IN) and was then fractionated by electrophoresis on an agarose gel (1%).

After denaturation in alkaline solution the DNA was transferred to a Nytran filter by capillary

acti n. The banding pattern obtained was m st consistent with n c py p r haploid g nome f a small gene encoding the IL-8 r cept r (Fig. 4). Detection of faint bands, however, in DNA digested with Eco RV, Hind III, and Xba I after long exposure of the blot suggested that another human homologue of F3R could be found with the p2 probe. HL60 neutrophil cDNA libraries were therefore rescreened with a p2 probe. Thirteen hybridizing plaques were sequenced; all were identical to p2. Therefore, a gene encoding a receptor more closely related to F3R is expressed either at very low levels, or not at all, in HL60 neutrophils.

Characteristics of various portions of the IL-8 receptor have been established. It has been found that the receptor contains seven hydrophobic segments predicted to span the cell membrane, a characteristic of the superfamily of G protein-coupled receptors (Fig. 3). The COOH-terminal segment contains 11 serine or threonine residues that may be phosphorylation sites for cellular kinases. The 20 amino acid third cytoplasmic loop, which may interact with G proteins, is similar in size to that of other peptide receptors. The IL-8 receptor has a single predicted site for N-linked glycosylation in the NH_2-terminal segment and two sit s in the sec nd extracellular l p. As with the C5a recept r, th NH_2-terminal segment is rich in

acidic residues and may form the binding site for IL-8, which is basic (pI-9.5). See N. P. Gerard and C. Gerard, Nature 349, 614 (1991).

The IL-8 receptor has been compared with other related receptors. The IL-8 receptor possesses 69% amino acid identity to F3R after the imposition of 10 gaps. If only the predicted transmembrane domains (TMD) are compared, 84% identity is found with F3R. Alignment with ten other G protein-coupled receptor sequences and examination of corresponding DNA sequences indicates that the apparent divergence of the IL-8 receptor from F3R between residues 92 and 105 is due to a frame shift in F3R. See T. I. Bonner, A. C. Young, M. R. Brann, N. J. Buckley, Neuron 1, 403 (1988); and S. Yokoyama, K. E. Eisenberg, A. F. Wright, Mol. Biol. Evol. 6, 342 (1989). Moderately conserved domains include the NH_2-terminal segment (38% identity, 4 gaps), the first extracellular loop (33%, 1 gap) and the COOH-terminal 23 residues (22%, no gaps). The third cytoplasmic loops are 95% identical. The IL-8 receptor possesses less than 30% amino acid identity with all other reported G protein-coupled receptor sequences including that of FPR (Fig. 3).

In a related development, a cDNA from human neutrophils has been found that encodes a distinct IL-8 receptor. This receptor has 77% amino acid identity with the IL-8 receptor encoded by p2, and

is more closely related to F3R (79% versus 69% amino acid identity). Neither human IL-8 receptor interacts with N-formyl peptides. The receptor encoded by p2 diverges most extensively from the other two sequences in the NH_2-terminal segment, although the acidic character of this region is conserved. Thus, the human neutrophil expresses at least two distinct calcium mobilizing IL-8 receptors. One of these, that encoded by p2, also is a gro receptor. The ability to bind gro of the other IL-8 receptor, the one more closely related to F3R, is not known. Structural comparison of the human IL-8 receptors with F3R predicts that F3R encodes a high affinity rabbit IL-8 receptor. In fact, to corroborate this prediction, Thomas et al. recently reported that F3R does encode a high affinity rabbit IL-8 receptor (See K.M. Thomas, L. Taylor, J. Navarro, J. Biol. Chem., 266, 14839-14841 (1991)).

While the foregoing invention has been described in some detail for purposes of clarity and understanding, it will be clear to one skilled in the art from a reading of this disclosure that various changes in form and detail can be made without departing from the true scope of the invention. All publications cited in this application are specifically incorporated by

referenc herein.

WHAT IS CLAIMED IS:

1. An int rleukin-8 r ceptor wher in said r cept r has an amino acid s quence as shown in Figure 3.

2. A DNA segment encoding the receptor of claim 1 wherein said receptor has a nucleotide sequence as shown in Figure 5.

3. A substantially pure form of the interleukin-8 receptor according to claim 1.

4. An interleukin-8 receptor wherein said receptor has greater than 77% homology with an amino acid sequence shown in Figure 3 and is a gro receptor.

5. A recombinant DNA molecule comprising:

a) said DNA segment according to claim 2; and

b) a vector for introducing said DNA into host cells.

6. The recombinant DNA molecule according to claim 5 wherein said vector is pcDNAI.

7. The receptor of claim 1 wherein said receptor has intracellular calcium mobilizing properties and ligand binding properties.

8. A host cell stably transfected with the recombinant DNA molecule of claim 1 in a manner allowing expression of a functionally active form of said protein encoded by said DNA segment.

9. The host cell of claim 8 wherein said host cell is a COS cell.

10. A method of producing an Interleukin-8 protein, said protein having an amino acid sequence as shown in Figure 3 comprising the steps of

culturing host cells according to claim 8 in a manner allowing expression of said protein and

isolating said protein from said host cells.

11. An RNA molecule transcribed from the DNA segment of claim 2.

12. An oocyte containing the RNA molecule of claim 11 in a manner allowing expression of a functionally active form of said receptor encoded by said DNA segment.

13. Th oocyte of claim 12 wherein said cyt is a *Xenopus laevis* ocyt .

14. A method of producing an interleukin-8 receptor protein in an oocyte comprising the steps of

injecting an oocyte with the RNA molecule of claim 11,

expressing said DNA segment in said oocyte,

and isolating said protein from said oocyte.

16. A method of detecting the presence or absence in a sample of a DNA segment encoding interleukin-8 receptor or a related MIP-2 receptor protein comprising the steps of

contacting said sample with a DNA probe comprising at least a portion of said DNA segment of claim 2 under conditions such that hybridization between said probe and said DNA segment of said sample occurs, and

detecting the presence or absence of a complex formed between said probe and said DNA segment.

17. A method of screening a ligand for the IL-8 receptor according to claim 1 comprising the steps of

contacting said ligand to the receptor under conditions such that binding to the receptor can occur,

measuring binding affinity of the ligand to the receptor and

measuring calcium flux associated with the binding of the ligand to the receptor.

18. The method according to claim 17 wherein the receptor is in the oocyte according to claim 12.

19. The method according to claim 16 wherein the receptor is in the host cell according to claim 8.

FIG. IA

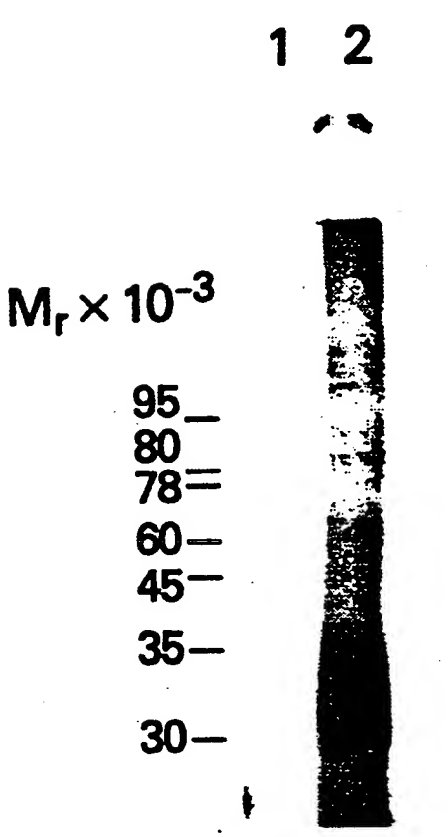

FIG. IB

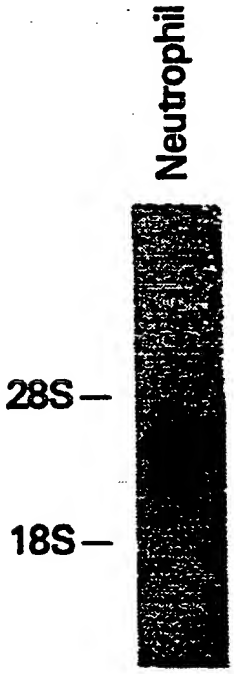

FIG. IC

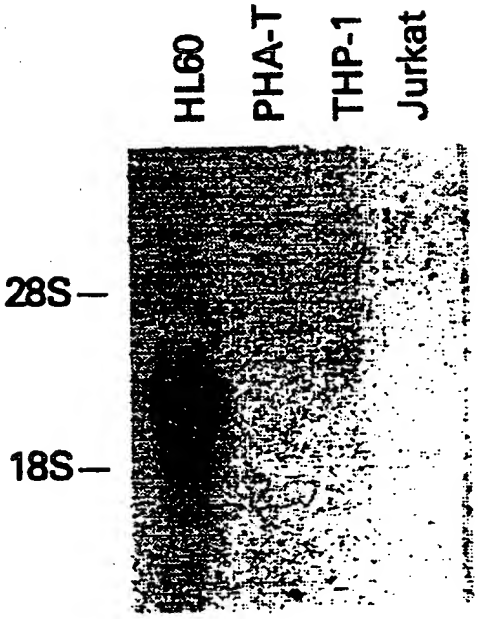

FIG. 2C

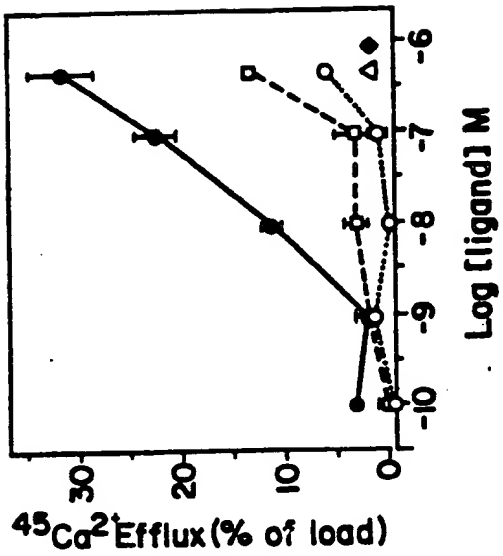

FIG. 2B

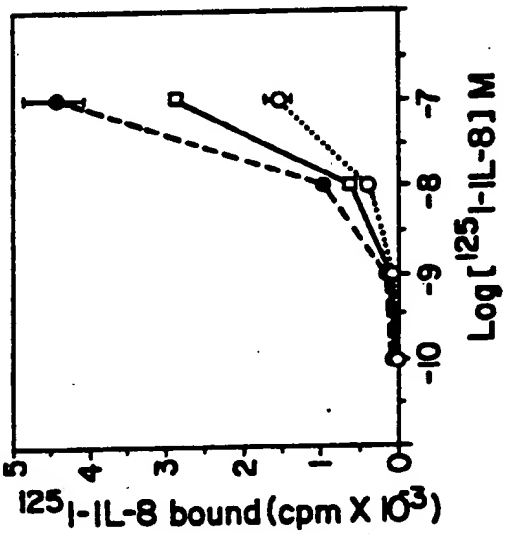

FIG. 2A

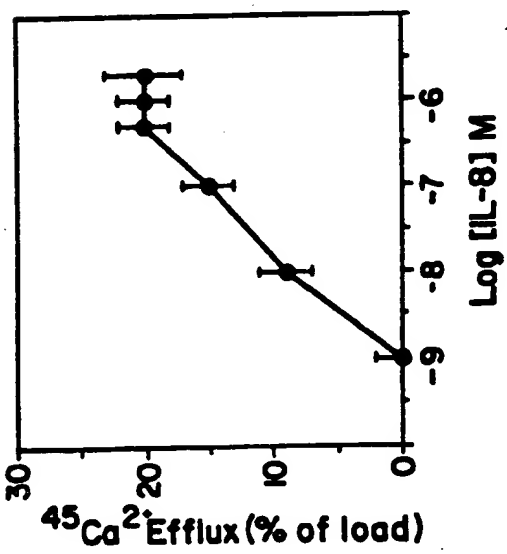

FIG. 3A

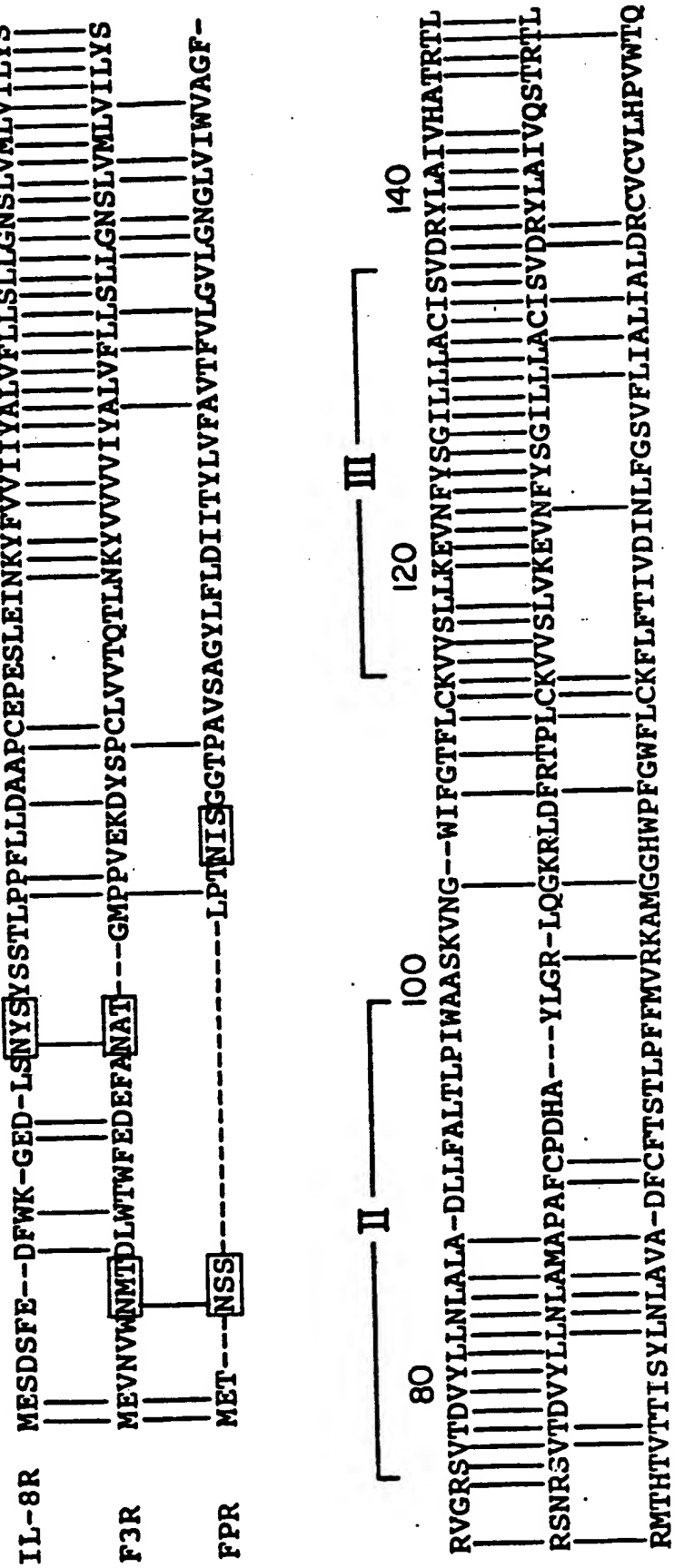
I
II
III
IL-8R
F3R
FPR
MESDSFE--DFWK-GED-LSNYSYSSTLPPFLLDAAPCEPESLEINKYFVVIIYALVFLLSLLGNSLVMLVILYS
MEVNVWNMTDLWTWFEDEFANAT--GMPPVEKDYSPCLVVTQTLNKYVVVIYALVFLLSLLGNSLVMLVILYS
MET---NSS----------LPTNISGGTPAVSAGYLFLDIITYLVFAVTFVLGVLGNGLVIWVAGF-
RVGRSVTDVYLLNLALA-DLLFALTLPIWAASKVNG--WIFGTFLCKVVSLLKEVNFYSGILLLACISVDRYLAIVHATRTL
RSNRSVTDVYLLNLAMAPAFCPDHA---YLGR-LQGKRLDFRTPLCKVVSLVKEVNFYSGILLLACISVDRYLAIVQSTRTL
RMTHTVTTISYLNLAVA-DFCFTSTLPFFMVRKAMGGHWPFGWFLCKFLFTIVDINLFGSVFLIALIALDRCVCVLHPVWTQ
20
40
60
80
100
120
140

FIG. 3B

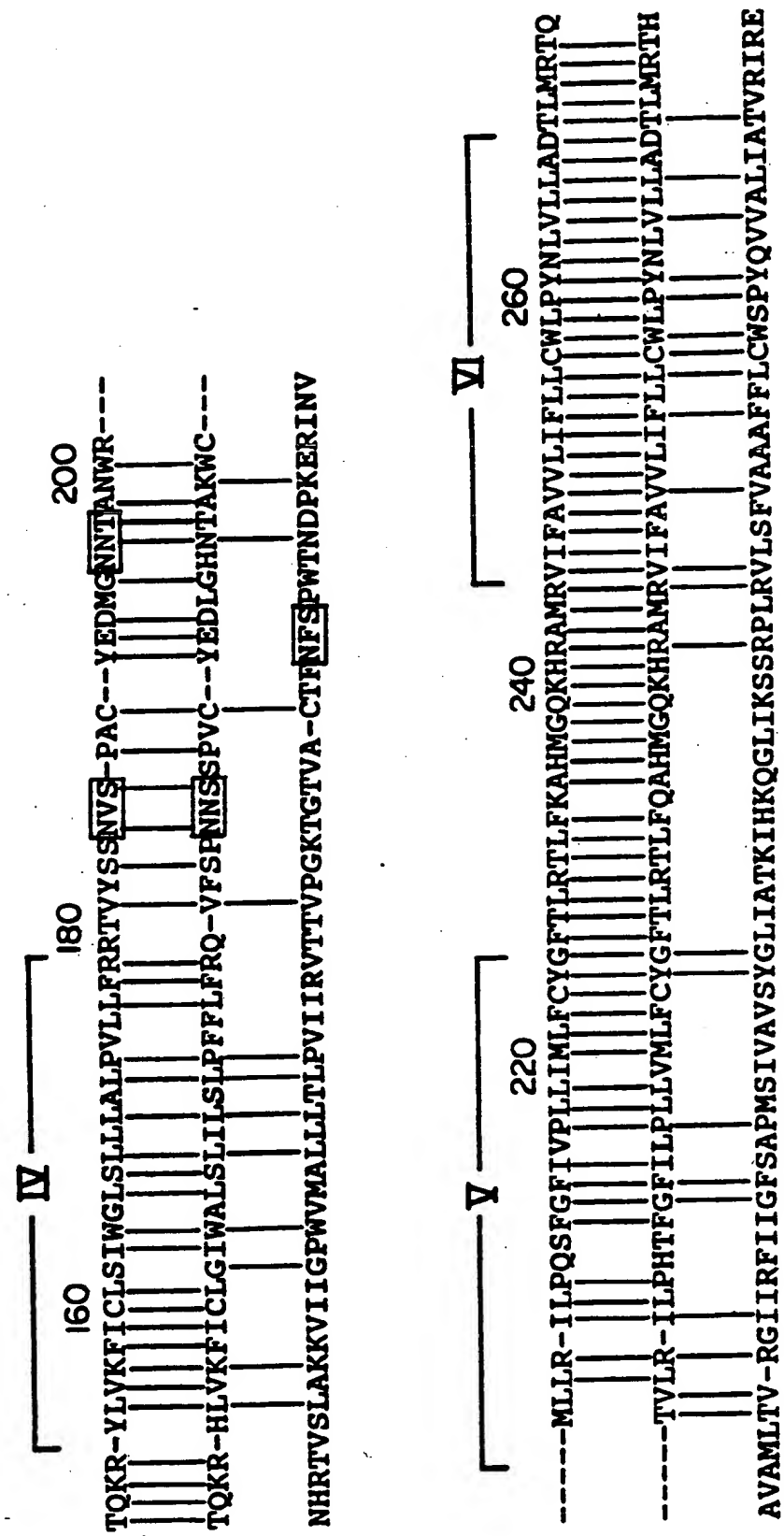
IV
160
180
200
TQKR-YLVKFICLSIWGLSLLLALPVLLFRRTVYSSNVS-PAC--YEDMGNNTANWR---
TQKR-HLVKFICLGIWALSLILSLPFFLFRQ-VFSPNNSSPVC--YEDLGHNTAKWC---
NHRTVSLAKKVIIGPWVMALLLTLPVIIRVTTVPGKTGTVA-CTFNFSPWTNDPKERINV
V
220
VI
240
260
-----MLLR-ILPQSFGFIVPLLIMLFCYGFTLRTLFKAHMGQKHRAMRVIFAVVLIFLLCWLPYNLVLLADTLMRTQ
-----TVLR-ILPHTFGFILPLLVMLFCYGFTLRTLFQAHMGQKHRAMRVIFAVVLIFLLCWLPYNLVLLADTLMRTH
AVAMLTV-RGIIRFIIGFSAPMSIVAVSYGLIATKIHKQGLIKSSRPLRVLSFVAAAFFLCWSPYQVVALIATVRIRE

FIG. 3C

280
300
320
340
VII
VIQETCERRNHIDRALDATEILGILHSCLNPLIYAFIGQKFRHGLLKILAI---HGLISKDSLPKDSRPSFVGSSSGHTSTTL 355
VIQETCQRRNELDRALDATEILGFLHSCLNPIIYAFIGQNFRNGFLKMLAA---RGLISKEFLTRHRVTSYTSSSTNVPSNL 354
LLQGMY---KEIGIAVDVTSALAFFNSCLNPMLYVFMGQDFRERLIHALPASLERALTEDSTQTSDTATNSTLPSAEVALQAK 350

FIG. 4

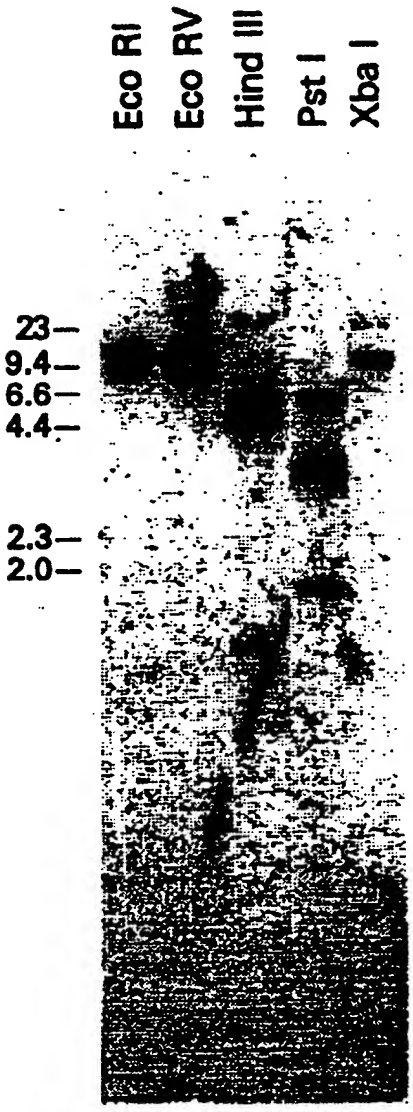

FIG. 5A

```
  1  GTCAGGATTT AAGTTTACCT CAAAAATGGA AGATTTTAAC ATGGAGAGTG

 51  ACAGCTTTGA AGATTTCTGG AAAGGTGAAG ATCTTAGTAA TTACAGTTAC

101  AGCTCTACCC TGCCCCCTTT TCTACTAGAT GCCGCCCCAT GTGAACCAGA

151  ATCCCTGGAA ATCAACAAGT ATTTTGTGGT CATTATCTAT GCCCTGGTAT

201  TCCTGCTGAG CCTGCTGGGA AACTCCCTCG TGATGCTGGT CATCTTATAC

251  AGCAGGGTCG GCCGCTCCGT CACTGATGTC TACCTGCTGA ACCTAGCCTT

301  GGCCGACCTA CTCTTTGCCC TGACCTTGCC CATCTGGGCC GCCTCCAAGG

351  TGAATGGCTG GATTTTTGGC ACATTCCTGT GCAAGGTGGT CTCACTCCTG

401  AAGGAAGTCA ACTTCTATAG TGGCATCCTG CTACTGGCCT GCATCAGTGT

451  GGACCGTTAC CTGGCCATTG TCCATGCCAC ACGCACACTG ACCCAGAAGC
```

FIG. 5B

501 GCTACTTGGT CAAATTCATA TGTCTCAGCA TCTGGGGTCT GTCCTTGCTC

551 CTGGCCCTGC CTGTCTTACT TTTCCGAAGG ACCGTCTACT CATCCAATGT

601 TAGCCCAGCC TGCTATGAGG ACATGGGCAA CAATACAGCA AACTGGCGGA

651 TGCTGTTACG GATCCTGCCC CAGTCCTTTG GCTTCATCGT GCCACTGCTG

701 ATCATGCTGT TCTGCTACGG ATTCACCCTG CGTACGCTGT TTAAGGCCCA

751 CATGGGGCAG AAGCACCGGG CCATGCGGGT CATCTTTGCT GTCGTCCTCA

801 TCTTCCTGCT TTGCTGGCTG CCCTACAACC TGGTCCTGCT GGCAGACACC

851 CTCATGAGGA CCCAGGTGAT CCAGGAGACC TGTGAGCGCC GCAATCACAT

901 CGACCGGGCT CTGGATGCCA CCGAGATTCT GGGCATCCTT CACAGCTGCC

951 TCAACCCCCT CATCTACGCC TTCATTGGCC AGAAGTTTCG CCATGGACTC

FIG. 5C

```
1001  CTCAAGATTC TAGCTATACA TGGCTTGATC AGCAAGGACT CCCTGCCCAA

1051  AGACAGCAGG CCTTCCTTTG TTGGCTCTTC TTCAGGGCAC ACTTCCACTA

1101  CTCTCTAAGA CCTCCTGCCT AAGTGCAGCC CGTGGGGTTC CTCCCTTCTC

1151  TTCACAGTCA CATTCCAAGC CTCATGTCCA CTGGTTCTTC TTGGTCTCAG

1201  TGTCAATGCA GCCCCCATTG TGGTCACAGG AAGCAGAGGA GGCCACGTTC

1251  TTACTAGTTT CCCTTGCATG GTTTAGAAAG CTTGCCCTGG TGCCTCACCC

1301  CTTGCCATAA TTACTATGTC ATTTGCTGGA GCTCTGCCCA TCCTGCCCCT

1351  GAGCCCATGG CACTCTATGT TCTAAGAAGT GAAAATCTAC ACTCCAGTGA

1401  GACAGCTCTG CATACTCATT AGGATGGCTA GTATCAAAAG AAAGAAAATC

1451  AGGCTGGCCA ACGGGATGAA ACCCTGTCTC TACTAAAAAT ACAAAAAAAA

1500  AAAAAAAAAA
```

FIG. 6

5'-GACGTCTACCTGCTGAACCTGGCCATGGCACCTGCTTTT-3'